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Assisted reproduction,
congenital malformation and
imprinted genes – Is there
reason for concern?

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Current knowledge of malformations following IFV is preliminary

<u>Author</u>	<u>N</u>	<u>Major</u>	<u>minor</u>
Govaerts, 1998	141	0	1.4 %
Van Golde, 1999	119	1.6%	
Loft, 1999	721	3.5%	
Wennerholm, 2000	1139	4.1%	
Palmero, 2000	2059	1.1%	0.5 %
Lancaster, 2000	2762	2.5%	
Bonduelle, 2002	2840	3.4%	6.3%

Current knowledge

- Has risk been properly assessed?
- Is there a biologic reason to be concerned?

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
- 2) Inconsistencies in determination of congenital anomalies
- 3) Heterogeneity of ART - ovulation induction, IVF, ICSI, assisted hatching, etc

#1 Comparing infertility population to general population

- Etiology of infertility may be tied to congenital/genetic disease
- Infertile population may be different than naturally conceiving population by known and unknown covariants

Etiology of infertility as a factor - oligospermia

Author	Def.	N	Autosomal translocations	XX/XY anomalies	Total % abnormal
Mastuda	<20	326	11 (3.4%)	5(1.5%)	4.9%
Bourr.	<10	594	23 (3.9%)	11 (1.9%)	5.8%
Abylho.	<10	180	6 (3.3%)	5 (2.7%)	6.1%

Habitual miscarriage -balanced translocations

- Newborn surveys 0.2%
- Habitual SAB couples 3 - 4 %
- > 3 consecutive SABs 9.2 %

- infertile couples 0.6 %
- >10 IVF cycles failed 3.2 %

- Stern C., 1999

Male factor – congenital bilateral absence of the vas deferens

Author	N	Compound Heterozygote	Heterozygote	% CF carriers (at least one allele)
Mercier	67	24%	42%	66%
Chillon	102	19% - two mutations 34% - mutation/5T	25% - one mutation 7% - 5T alone	78% 85% (incl 5T)

Female factor infertility

- X/Autosomal translocations
- X chromosome mosaics
- Fragile X Premutation Carriers
- Single gene disorders

Chromosomal anomalies associated with ICSI conceptions likely reflect paternal condition

- 1.2% risk of chromosomal rearrangements
 - All of paternal origin
 - All present on paternal peripheral blood analysis
- 1.0% risk of sex chromosome aneuploidy
 - 4/5 cases determined to be of paternal origin
 - No evidence of paternal mosaics in peripheral blood (Bonduelle, 1996; In't Veld, 1996)

Is the infertile population potentially different ?

- Known and unknown covariates of infertility and congenital malformations
 - Maternal and paternal age differences
 - Exposures - smoking
 - Prenatal care – folate, etc
 - Pregnancy termination

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
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#2 Determination of congenital anomalies - controls usually from “registry”

- Completeness of exam variable
- Increased use of ultrasound in infertility patients and follow-up of identified concerns
- Timing of neonatal exam
 - 2-3% rate is in first 48 hours

Definition of anomaly

- Major versus minor
- Coding of anomaly
- Single versus multiple

Sample sizes and power for events of low frequency

- 3% rate requires 244 cases to have 95% confidence to detect a 3 fold increase
- Requires pooling of anomalies which may dilute a potential increase in a specific system or type of anomaly

Sample sizes and power for events of low frequency

- subset analyses fall victim to “multiple testing “
- testing 20 categories of anomalies would result in 1 reaching significance just due to multiple testing (5% for 20)

Assessment of risk - potential pitfalls

- 1) Use of poorly delineated study populations
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- 3) Heterogeneity of ART - ovulation induction, IVF, ICSI, assisted hatching, etc

#3 Heterogeneity of treatments not addressed

- IVF - per cent ICSI varies
 - Most recently 9% in European studies, 40% by CDC in 1999
- Ovulation agents; culture media; assisted hatching
- Spermatozoa inseminations, oocyte transfers

Prospective, well designed cohort study is needed with:

- IVF population systematically assessed for AR variables – etiology of infertility, interventions
- Both populations assessed for potential confounders
 - Information obtained concurrently not based on recall
- Large Ns needed
- Same determination of anomalies in both populations

Netherlands study, Anthony 2002

- IVF (N=4224) compared to natural conceptions (314,605)
 - Congenital malformations ascertained from registry used for all births
 - Birth registry interrogated by IVF registry and coded conception type

Netherlands study, Anthony 2002

- IVF population 3.2% major malformation
- General population 2.7%
- Odds ratio 1.20 (95% CI: 1.01-1.43)
- Corrected OR 1.03 (95% CI:0.86-1.23)
 - Maternal age, parity and ethnicity

Netherlands study, Anthony 2002

- Categories of malformation (9)
- Odds ratio below 1.0
 - Skin/abdominal wall
 - Chromosomal
- Odds ratio above 1.0
 - CNS
 - CVS OR=1.56 (95%CI: 1.10-2.22)
 - Digestive
 - Respiratory
 - Urogenital
 - Skeletal/muscular

Netherlands study, Anthony 2002

- Increase in congenital malformations attributable to difference in populations (maternal age, parity, ethnicity)
- When investigated by category, cardiovascular malformations are significantly increased
 - 7/9 categories had odds ratios greater than 1.0

Netherlands study, Anthony 2002

How reliable are the registries ?

Subset of 1716 IVF children with additional questionnaire data

- * 95 with congenital malformations not recorded
- * 16/95 should have been recorded
- * 17% under-reporting in registry

Australian study by Hansen in NEJM March 2002

- Similar and well delineated cases and general population
 - same source of data for both populations
 - only 3 centers for over 1000 AR infants (301 ICSI, 837 IVF alone)

Australian study by Hansen in NEJM March 2002

- same definition of birth defects and same time frame (to one year)
- only majors
- essentially all pregnancies get a 16-20 week US in Western Australia

Australian study by Hansen in NEJM March 2002

major anomaly by one year of age

ICSI	26/301	8.6 % (5.7-12.4%)
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IVF	75/837	9.0 % (7.1-11.1%)
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General

Population	168/4000	4.2 % (3.6-4.9%)
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Australian study by Hansen

NEJM, March 2002

- results held
 - only singletons studied
 - only singletons at term
 - adjusted for maternal age, parity, sex of the infant
 - independent exclusion of malformations possibly diagnosed early because of increased surveillance (either population)

Australian study by Hansen, March 2002

	Natural	ICSI	IVF
Tabs included	4.5%	8.6%	9.5%
Genetic excluded	4.0	8.0	8.5
Minors excluded	4.0	8.1	8.9
Chromosomal excluded	4.0	8.1	8.9
Multiple defects	0.5	2.0	1.6

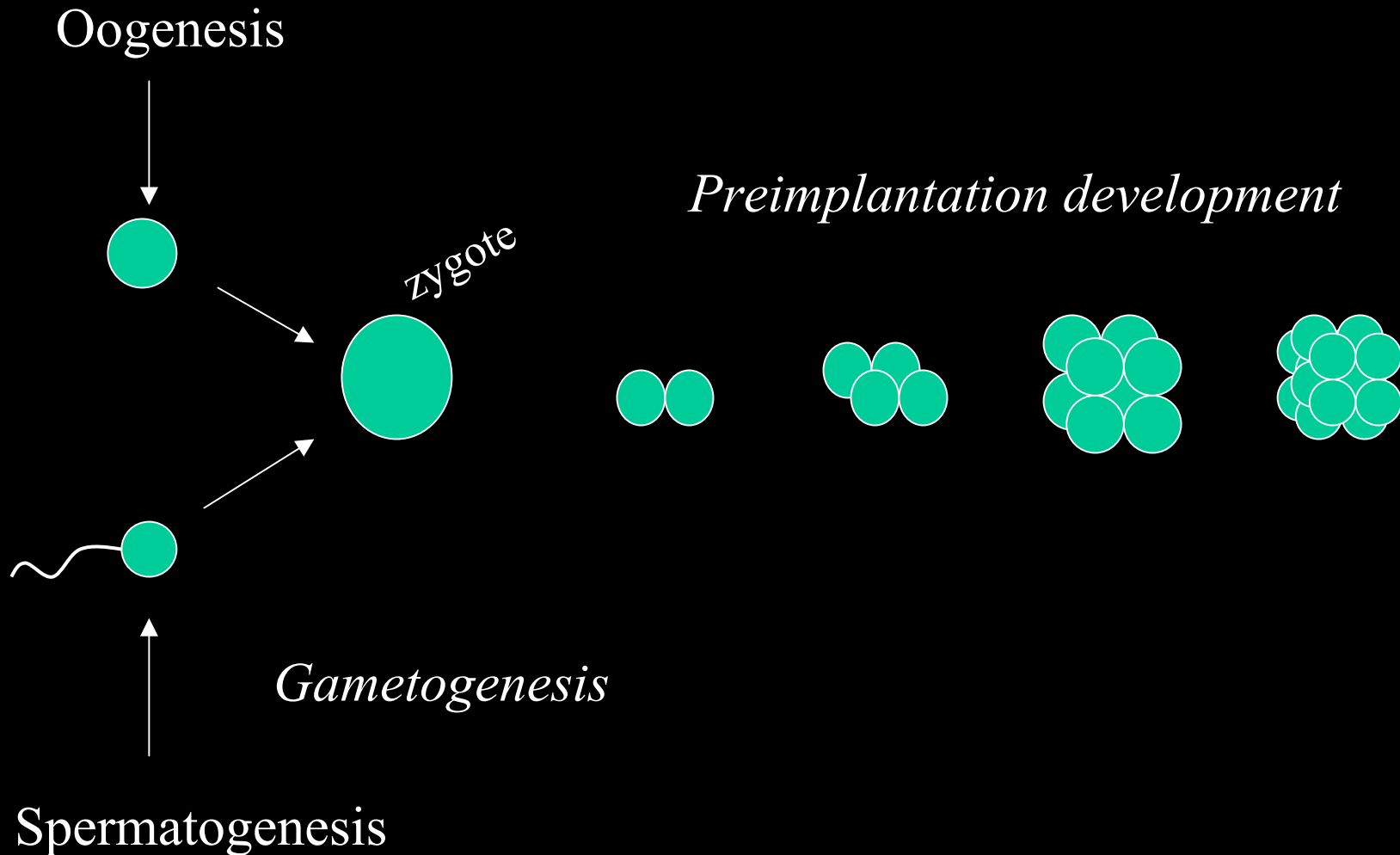
Australian study

- More study is needed
- A small increase, perhaps doubling is probably present
- Issue of multiple anomalies raised
- Due to IVF/ICS or some also to intrinsic to characteristics of infertile population

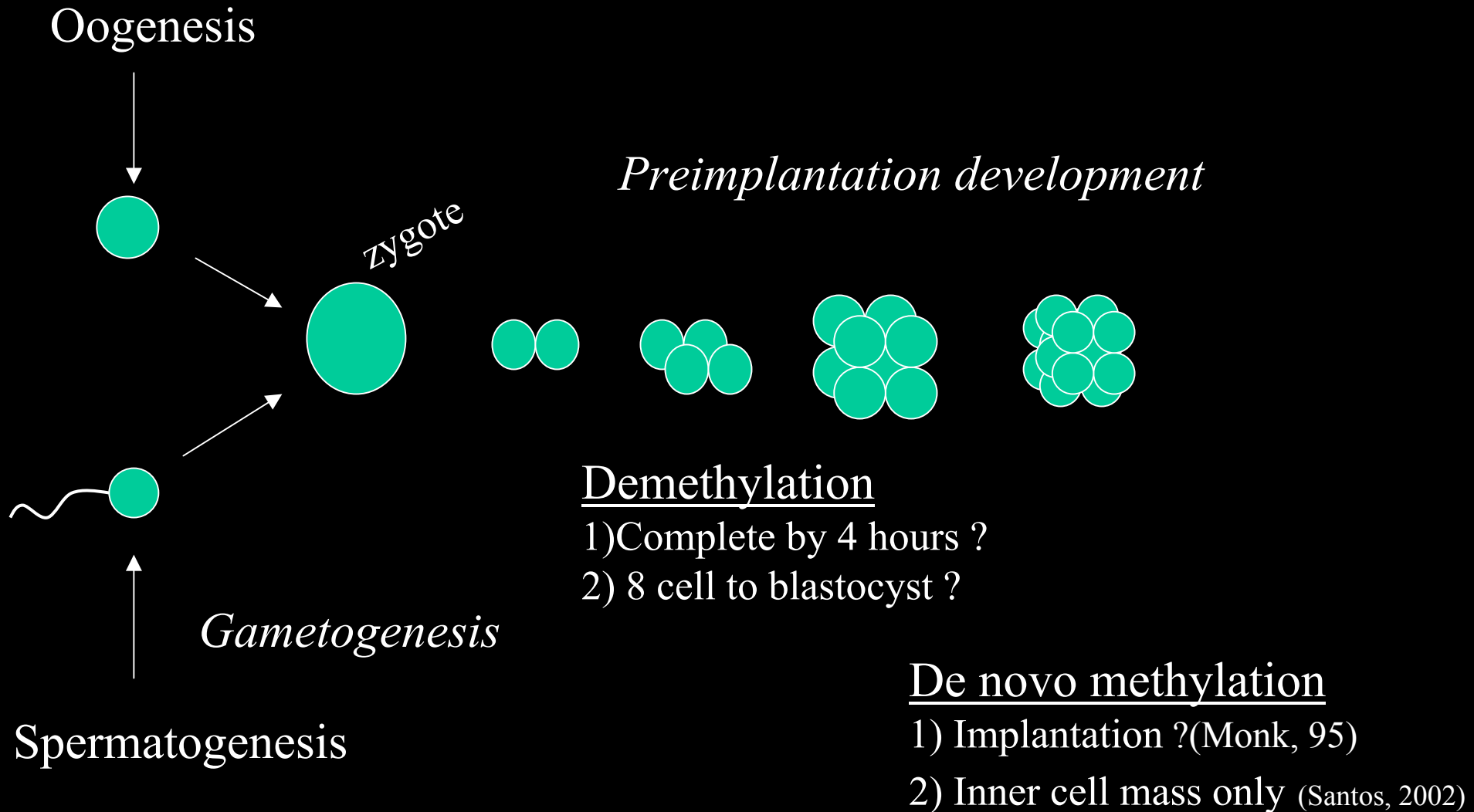
Is there a biologically plausible concern regarding genetic damage?

- Both gametogenesis and preimplantation are times of epigenetic mechanisms
 - Alterations in DNA structure rather than sequence
 - Turning on/off of embryonic genes

Potential sites of imprinting



Assisted reproduction and imprinting



Controls of imprinting

- Germline
 - Cis regulatory elements direct allele specific imprinting
 - DMRs –differential methylation regions
- Postfertilization
 - DMRs retained despite genome wide demethylation and de novo methylation
 - Demethylation in morula stage
 - De novo methylation in pregastrula (Kafri,1993)

Control of methylation

- Dnmt1 methyltransferase
 - Synthesized in oocyte cytoplasm
 - Maintains DNA methylation of imprinted alleles
 - Traffics to nucleus at 8 cell stage only
 - Implies other methyltransferases needed

Timing of imprinting (Ohno, 2001)

- Mouse imprinted genes – conflicting evidence for monoallelic transcription in preimplantation embryos
- ASO-RNA-FISH
- Igf2
 - First detected at 2 cell stage
 - Biallelic up to morula
 - Maternal allele silenced during blastocyst
 - In vitro cultured showed bias toward maternal transcription up to morula

Timing of imprinting (Monk, 2001; Salpekar, 2001)

- Human imprinted genes – variability in onset of expression which is timing and tissue specific, also variable from embryo to embryo
- cDNA from oocytes, 4, 8, blastocyst stages
 - SNRPN, PEG1, UBE3A each expressed
 - Only SNRPN expressed from monoallele (paternal copy)
 - SNRPN monoallelic by 4 cell stage (Huntriss, 1998)

Can imprints be altered ? (Doherty, 2000)

- In mice, monoallelic versus biallelic expression of H19 dependent on media
 - Standard versus supplemented with amino acids
- Biallelic expression concordant with loss of methylation
- Not all imprinted genes affected
 - Snrpn not altered in same media system

Can imprints be altered? (Khosia, 2001)

- Effect of serum addition to media
 - Controls – in vivo conception and implantation
 - Media alone – in vitro
 - Media with fetal calf serum – in vitro
- 1/3 of blastocysts in supplemented media implanted and survived to day 14 fetuses

Timing of imprints (Khosia, 2001)

- Presence of fetal calf serum supplemented media
 - Smaller fetuses compared to both control groups
 - Decreased expression of H19 and IGF2
 - Increased methylation at imprinting center of H19

Any relationship between IVF and imprinting disorders?

- 100 children with the majority conceived by IVF with ICSI with ejaculated sperm (Manning 2000)
 - no imprinting defects of chromosome 15

Any relationship between IVF and imprinting disorders?

- Angelman syndrome and ICSI (Cox, 2001)
 - Imprinting defect occurs in $< 5\%$ of cases
 - Maternal allele is unmethylated
 - Case report of two children conceived with ICSI
 - Isolated imprinting defect in both
 - 1/300,000

Any relationship between IVF and imprinting disorders - Beckwith Wiedemann

- LGA, macroglossia, omphalocele
- overgrowth syndromes - omphalocele, CDH
 - altered closures due to organomegaly
 - increased risk of malignancy

Association of BWS with ICSI

(DeBaun, 2003)

- Registry of BWS since 1994

	IVF conceptions	ICSI
Registry	4.6 %	70%
1999 (CDC)	0.76%	42%

- At least a 6 fold increase in BWS

Association of BWS with ICSI

(DeBaun, 2003)

- 5/6 children had imprinting errors of LIT1
 - Maternal allele DMR hypomethylated
 - Generally 60% of BWS due to LIT1
 - Without LIT1 imprint (methylation), LIT1 expression increased suppressing other genes in region

Association of BWS with ICSI

(DeBaun, 2003)

- 1/6 children had abnormal imprinting of LIT1 and H19
 - Hypermethylation of H19 DMR
 - 15% with H19 imprinting errors
- 1/6 children had no aberrant methylation

Any relationship between IVF and imprinting disorders - Large offspring syndrome (LOS) in cattle

- Cattle and sheep with LGA at birth, congenital malformations
 - Can occur in 1/3 of in vitro conceptions
- Young and Fiarburn, 2000 - theorized as due to problems in imprinted genes --- epigenetic misprogramming

Emerging information on LOS and imprinting in animals

- LOS in sheep associated with reduced expression of Igf2r (Young, 2001)
 - through loss of methylation following embryo culture
 - locus not imprinted in humans

In vitro compared to in vivo cultures (Niemann, 2000)

- Altered levels of expression
 - Heat shock protein
- Transcription of some genes lacking
 - Connexin43 gene (crucial for maintenance of compaction)
- New transcription of other genes
 - bovine leukemia inhibitory factor (bLIF) and LIF-receptor-beta (LR-beta) genes

LOS produced in sheep (Lazzarri, 2002)

- In vitro culture with supplemented media
 - Increased number of cells in blastocysts
 - Increased overall size of blastocysts
 - Increased transcripts for developmentally important genes Hsp70.1, Cu/Zn-SOD, Glut-3, Glut-4, bFGF, and IGF1
 - Correlated with LOS in offspring

Where does this leave investigations of congenital malformation?

- Risk of congenital malformation following assisted reproduction may equal a doubling of background risk
- Potential genetic causes resulting in infertility and malformations need to be addressed
 - Oligospermia/chromosomal abnormalities
 - CBAVD and cystic fibrosis
- Alterations in imprinted genes are suspected based on animal LOS studies and initial human studies of Beckwith Wiedemann